Fluorescence-based assay development to screen drugs against Amyotrophic Lateral Sclerosis disease

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Abstract

Amyotrophic Lateral Sclerosis disease (ALS) is characterized by the death of both upper and lower motor neurons in the motor cortex of the brain, the brain stem, and the spinal cord. Prior to their destruction, motor neurons develop intracellular protein inclusions in their cell bodies and axons. These inclusions often contain ubiquitin, and generally incorporate one of the ALS-associated proteins: SOD1, TAR DNA binding protein (TDP-43, or TARDBP), or FUS. Innoprot has developed a novel fluorescence cell-based assay for High Content Screening to screen compounds that can prevent or decrease the protein TDP43 or FUS aggregation into the stress granules after induction of a cytotoxic stress conditions. In this work we used both models to screen a library of 1200 compounds. Arimoclomol and Riluzole compounds were used as positive controls for the fluorescent TDP43 and FUS aggregation model. After the screening campaign, positive compounds were chosen for further testing, based on the strength of the initial response and the lack of cytotoxicity. Our results indicated that the pharmacological inhibition or modulation TDP43 or FUS aggregation implicated in ALS is a valid strategy for drug screening.

Results

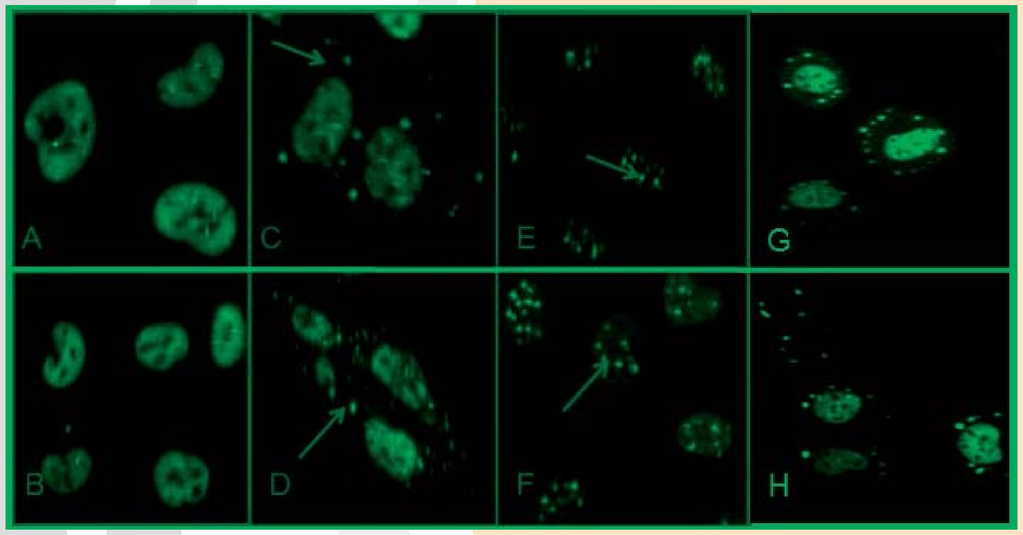

Fig.1. Cellular fluorescence redistribution after sodium arsenite treatment. Representative images of the negative controls show a nuclear distribution of the fluorescence (A,B). However, after sodium arsenite treatment the TDP43 (C,D) and FUS (G,H) phenotype turns into a cytosolic vesicular pattern corresponding to stress granules. In addition the TDP43 phenotype shows an intensive nuclear globs pattern (E,F).

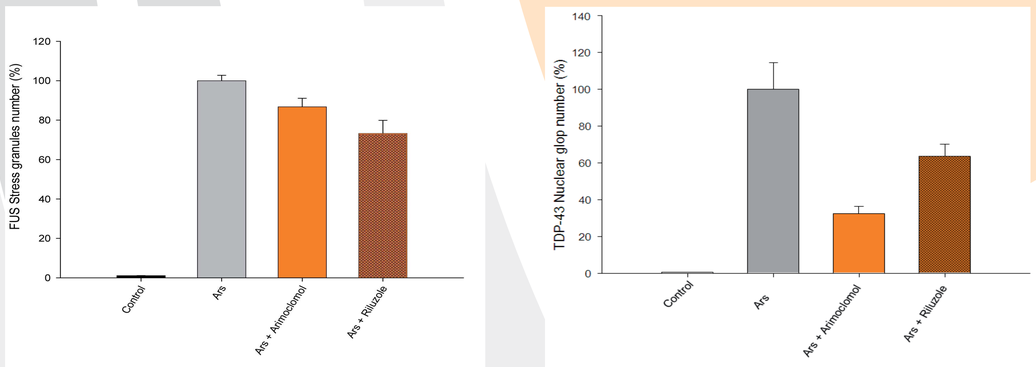

Fig.2. Protective effect of Aromoclimol and Riluzole againts cellular oxidative stress. TDP-43-tGFP and FUS-tGFP cellular models were incubated with Arimoclomol at 10 uM or Riluzole at 5 µM during 24 hours. Then, cells were treated with 250 µM sodium arsenite during 90 min. The TDP43-tGFP nuclear globs and the FUS citosolic granules were quantified using the BD Pathway HCS Reader and Attovision Compartimentalization Software. Error bars represent the standard deviation among 3 replicate wells.

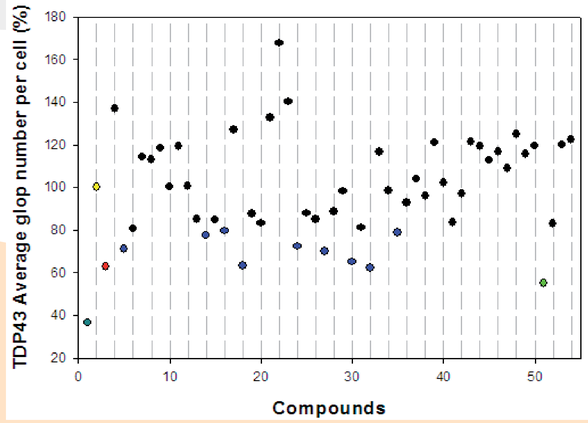

Fig.3. Screening of 57 compounds using the TDP43-tGFP model. Before oxidative stress induction by sodium arsenite, cells were incubated with the compounds at 10 µM during 24 hours. Then TDP43 nuclear glob number was quantified using Attovision software . The control of TDP43-tGFP expressing cells is represented in green. The positive control (Arimoclomol) is represented in red and the negative control (Sodium arsenite) is represented in yellow. The Blue spots represent compounds that show a nuclear globs numbers reduction around 20-40%. The light-green spots represent compounds that show a nuclear globs numbers reduction >40%.

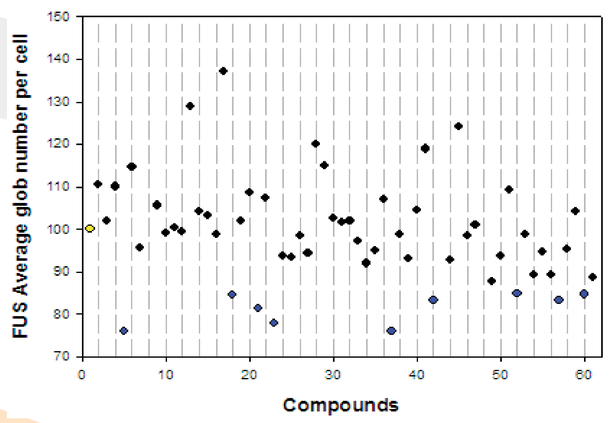

Fig.4. Screening of 57 compounds using the tGFP-FUS model. Before oxidative stress induction by sodium arsenite, the cells were incubated with the compounds at 10 µM during 24 hours. Then FUS granules number was quantified using Attovision software .The negative control (Sodium arsenite) is represented in yellow. The Blue spots represent compounds that show a nuclear globs numbers reduction around 10-20%.

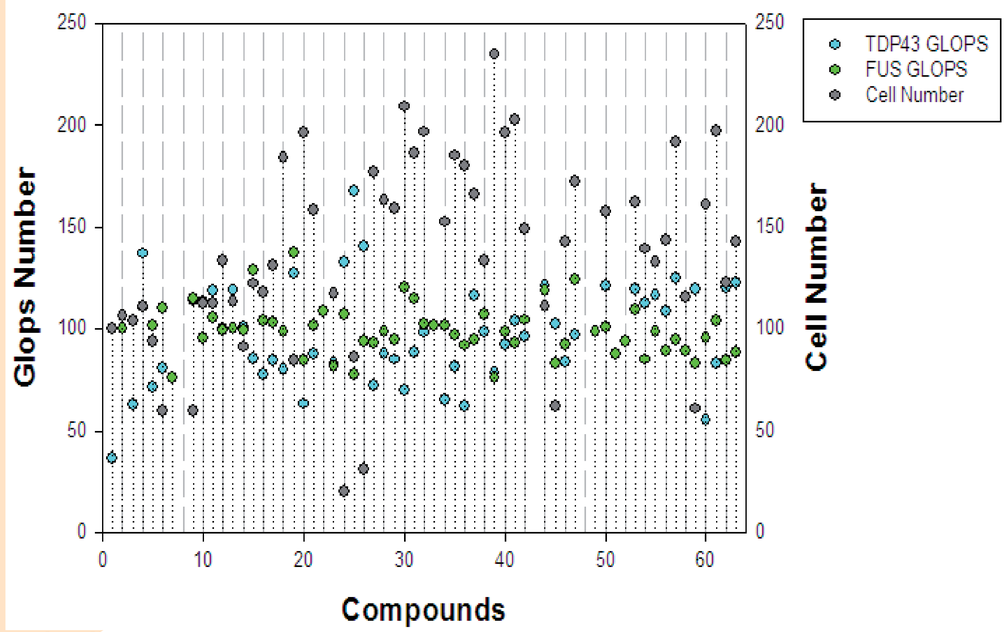

Fig.5. Compounds toxicity. The number of TDP43 globs (blue) and the number of FUS granules (green) are represented related to the cell number (grey). The results show the compounds that did not affect the cell viability, so they were selected for further studies.

Methods

Cultured cells: The U2OS cell line has been used for constitutive and inducible cell line generation. The cell line was cultured into 96 wells Imaging Plates BD at 4000cell/well in 200 µl of DMEM F12 10% FBS and incubated at 37 ºC and 5 % CO2.

Image acquisition: Cell lines stably expressing human tagged FUS or TDP43 proteins were treated with Sodium arsenite during 90 min. After that, the TDP43 and FUS inclusions presence was quantified by fluorescence using image analysis algorithms.

Conclusions

1.-The stably transfected TDP43 cell line and FUS cell line can be used in drug discovery for pathological globs formation inhibitors.
2.-These model permits evaluating the TDP-43 and FUS proteins distribution in living cells by the study of the protein localization pattern in the space and time.
3.-These models provide a strategy to evaluate drugs that not have cell permeability.
4.- These cellular models have been adapted to HCS analyses based on image algorithms to test cytosolic and nuclear globs generation process.